

WHAT IS CLAIMED IS:

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BR 1. A purified thermostable enzyme that catalyzes combination of nucleotide triphosphates to form a nucleic acid strand complementary to a nucleic acid template strand. ✓

5 2. The enzyme of claim 1 that is DNA polymerase.

3. The enzyme of claim 2 having a molecular weight of about 86-95,000 daltons.

4. The enzyme of claim 2 from a Thermus species.

5. The enzyme of claim 3 from Thermus aquaticus.

10 6. The enzyme of claim 1 having at least 50% of the activity at pH 6.4 that it has at pH 8.0.

7. The enzyme of claim 2 having at least 50% of the activity at pH 6.4 that it has at pH 8.0.

8. The enzyme of claim 1 that is in native form.

15 9. The enzyme of claim 2 that is in native form.

10. The enzyme of claim 3 that is in native form.

11. The enzyme of claim 4 that is in native form.

12. The enzyme of claim 5 that is in native form.

13. The enzyme of claim 6 that is in native form.

14. A purified native thermostable DNA polymerase from Thermus aquaticus having a molecular weight of 86-95,000 daltons and having at least half of the activity at pH 6.4 that it has at pH 8.0.

15. A gene encoding the enzyme of claim 1.

5 16. A gene encoding the enzyme of claim 4.

17. The gene of claim 15 that was cloned from the genome of Thermus aquaticus.

18. The gene of claim 17 that has the DNA sequence of Figure 1 or an allelic variant thereof.

10 19. The gene of claim 17 encoding a polymerase having a molecular weight of about 86,000-95,000 daltons.

20. The gene of claim 19 encoding a polymerase having the amino acid residues of 4-832 of Figure 1.

15 21. The gene of claim 19 encoding a polymerase having the 832 amino acid sequence of Figure 1.

22. The gene of claim 17 encoding a polymerase having a molecular weight of about 60,000-65,000 daltons.

23. The gene of claim 22 encoding a polymerase having the amino acid residues 290-832 of Figure 1.

20 24. The thermostable enzyme of claim 1 that is a polymerase containing at least 50% homology to any contiguous stretch of nine or more amino acids shown in Figure 1.

25 25. The thermostable polymerase of claim 24 wherein said contiguous stretch of nine or more amino acids is selected from the following sequences:

- a) residues 190-204;
- b) residues 262-270;
- c) residues 569-587;
- d) residues 718-732;
- 5 e) residues 743-759; and
- f) residues 778-790.

26. The enzyme produced recombinantly from the gene of claim 15.

27. The enzyme of claim 26 which has a non-blocked amino
10 terminus.

28. The enzyme produced recombinantly from the gene of claim 16.

29. The enzyme produced recombinantly from the gene of claim 17.

30. The enzyme produced recombinantly from the gene of
15 claim 19.

31. The enzyme produced recombinantly from the gene of claim 22.

32. A gene encoding the enzyme of claim 24.

20 33. The enzyme of claim 24 which is produced recombinantly.

34. A recombinant DNA vector comprising the gene of claim 16, which vector is selected from the group consisting of plasmids pFC85 and bacteriophage CH35:Tag#4-2.

35. A stable enzyme composition comprising the enzyme of claim 1 in a buffer comprising one or more non-ionic polymeric detergents.

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36. A stable enzyme composition comprising the enzyme of claim 5 in a buffer comprising one or more non-ionic polymeric detergents.

37. The composition of claim 36 wherein the detergents are each present in a concentration of about 0.1% to about 0.5% volume/volume of the total composition.

38. The composition of claim 35 wherein the detergent is a polyoxyethylated sorbitan monolaurate, an ethoxylated nonyl phenol or a combination thereof.

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39. The composition of claim 37 wherein the detergent is a polyoxyethylated sorbitan monolaurate, an ethoxylated nonyl phenol or a combination thereof.

40. The composition of claim 39 wherein the buffer comprises glycerol, Tris-Cl, pH 8.0, ethylenediamine tetraacetic acid, dithiothreitol, a polyoxyethylated sorbitan monolaurate, an ethoxylated nonyl phenol, and gelatin.

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41. The composition of claim 40 wherein the buffer comprises 50% (volume/volume) glycerol, 20 mM Tris-HCl, pH 8, 0.1 mM ethylenediamine tetraacetic acid, 1 mM dithiothreitol, 0.5% volume/volume of a polyoxyethylated sorbitan monolaurate, 0.5% volume/volume of an ethoxylated nonyl phenol, and 200 µg/ml gelatin.

42. A method for purifying a thermostable polymerase which comprises treating an aqueous mixture containing the thermostable polymerase with a hydrophobic interaction support under conditions which promote hydrophobic interactions and eluting said thermostable

polymerase from said support with a solvent which attenuates hydrophobic interactions.

43. The method of claim 42 wherein the hydrophobic chromatographic support is Phenyl Sepharose.

5 44. The method of claim 42 wherein said hydrophobic interactions are provided by a buffer with an ionic strength corresponding to greater than or equal to 0.05 M NaCl.

10 45. The method of claim 44 wherein said hydrophobic interaction promotion conditions are provided using a buffer containing greater than or equal to 0.2 M ammonium sulfate.

46. The method of claim 42 wherein said elution solvent uses a 0-4 M urea gradient.

47. The method of claim 42 wherein the thermostable polymerase is DNA polymerase isolated from Thermus aquaticus.

15 48. The method of claim 42 wherein said thermostable polymerase is a recombinant enzyme.

49. The method of claim 48 wherein the aqueous mixture has previously been enriched in thermostable polymerase activity by heat treating the cell lysate.

20 50. The method of claim 49 wherein the heat treatment is conducted at temperatures in the range of at least 45°C to about 90°C.

51. A method for purifying a recombinant thermostable polymerase produced in a heat labile host cell which method comprises treating the cell lysate with temperatures in the range of at least
25 45°C to about 90°C and recovering the thermostable polymerase activity.

52. The method of ~~claim~~ claim 51 wherein said thermostable polymerase is from Thermus aquaticus.

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